Postharvest Storage of Blackberry Fruit Does Not Increase Antioxidant Levels

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Abstract

Blackberries (*Rubus* sp.) are a rich source of anthocyanins and other polyphenolic antioxidants. Because of their antioxidant properties, dietary polyphenolics have been associated with a reduced risk of various degenerative conditions including certain cancers and disease. A number of studies have been done to identify germplasm high in ORAC (oxygen radical absorbing capacity) in *Rubus* species. The present study was done to determine how the ORAC of blackberries was affected by fruit storage. Blackberries of five cultivars, originating from the University of Arkansas breeding program (all tetraploids), grown in Lane, Okla. and harvested in 1998 at the shiny black and dull black stages of ripeness, were held at 2 ^{o}C, 95% relative humidity for 7 days plus 2 days at 20 ^{o}C. Non-decayed berries were freeze-dried and powder of drupelet and receptacle tissue (no seeds) was extracted with acidified methanol. Samples were prepared for ORAC analysis using a COBAS-FARA II spectrofluorometric centrifugal analyzer. No significant differences were found between shiny and dull black fruit. ORAC values were highest in 'Kiowa' and lowest in 'Shawnee' fruit (4048 and 2690 µmol trolox/g freeze dried tissue, respectively). Values averaged for stored fruit were slightly lower than for fresh berries (3110 vs 3393 µmol trolox/g, respectively). These results indicate that the ORAC of these blackberry cultivars is not significantly increased at the latter stages of ripeness or by fruit storage at 2 ^{o}C.

INTRODUCTION

Small fruits have been found to be rich sources of phytochemicals, primarily antioxidants. These free radical scavengers have been found to prevent initiation of some cancers and effective in reducing risk of cardiovascular disease. Much work has been focused on blueberries, which contain ascorbic acid (vitamin C), anthocyanins, polyphenolics, and condensed tannins, all of which have been found to play positive roles in human health (Prior et al., 1998).

Oxygen radical absorbance capacity (ORAC) is a measure of the total antioxidant capacity of a food, including vitamin C, phenolics, anthocyanins, and polyphenolics. Because of the relative difficulty of the assay, the relationship of ORAC to other, more easily measured compounds, such as anthocyanins or total phenolics, is often studied to determine whether an alternative assay that can be readily applied across the food and plant sciences will accurately predict the ORAC of plant-based foods.

Total anthocyanin content has been shown to increase after storage at temperatures >0^{o}C in several small fruits (Kalt et al., 1999). In erect-type blackberries, anthocyanin content increases when fruit are stored at temperatures above 5^{o}C (Perkins-Veazie et al., 1999). Increases in anthocyanin are highly specific to cultivar, with 'Navaho' expressing more increases in anthocyanin than other cultivars. Wang and Lin (2000) studied the relationship of fruit maturity to ORAC in Eastern Thornless (semi-erect) blackberries. However, comparisons of ORAC among the erect-type blackberries commonly grown in the southern United States has not been done. This study was to determine the effects of storage on total anthocyanins, phenolics, and the ORAC of fresh erect-type blackberries.

Proc. 8th IS on Rubus and Ribes
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MATERIALS AND METHODS

Blackberries were grown at Lane, Oklahoma under standard growing conditions. No fungicides were applied from bloom onward. Berries were harvested three times within the fruiting period for each cultivar, at one to two week intervals (corresponding to 20% ripe fruit, 90% ripe primary/20% ripe secondary fruit, and 60% ripe secondary fruit, respectively). In Oklahoma, fruit of 'Arapaho' and 'Choctaw' ripen first, followed by 'Kiowa', 'Shawnee' and 'Navaho'. Fruit were placed in 160 g polyethylene vented clamshell boxes and stored at 2^{o}C within 1 h of harvest.

After 7 days storage at 2 ^{o}C, one half of the boxes were moved to 20^{o}C for 2 days to simulate retail conditions. All berries were rated for decay, leak, red drupe, and softness. Fruit with no visible decay were frozen at -80^{o}C then freeze dried. Freeze dried berries were crushed to a fine powder and 1 g without seeds was used for chemical analyses. Anthocyanin, phenolics, and antioxidants were extracted by adding 25 ml of methanol/acetone/water/acetic acid (40:40:20:0.1) to powder and stirring until color was completely extracted from tissue (about 15 min). Supernatants were dried under vacuum, held at -20^{o}C until analysis for ORAC, and rehydrated in one ml of water. Total anthocyanin and phenolic concentration were determined by measuring spectro-photometric absorbance at 520 and 280 nm wavelengths (Mazza et al., 1999). Total anthocyanin was calculated as mg cyanidin-3-glucoside (the primary anthocyanin in blackberries) using E=29,600, and total phenolics as mg gallic acid equivalents. ORAC was measured using the method of Cao et al. (1995), as described in Kalt et al. (1999).

RESULTS AND DISCUSSION

Of the variables tested, cultivar had the most effect on ORAC, total anthocyanin and total phenolics (Table 1). Across cultivars, holding berries an additional 2 days at 20^{o}C following 7 days storage at 2^{o}C had little effect on any of the variables. Berries harvested later in the fruiting season were slightly higher in ORAC than those harvested early in the season. Slightly unripe berries (shiny black) were similar in total anthocyanin, phenolics, and ORAC to fully ripe (dull black) berries. 'Kiowa' berries were higher in total anthocyanin, phenolics, and ORAC than the other cultivars.

Fruit values for stored fruit were averaged and compared against fresh fruit (Table 2). Total anthocyanin content decreased or stayed the same for 3 of the 4 cultivars tested. Total phenolics increased in stored 'Arapaho' and 'Shawnee' while ORAC decreased in stored 'Arapaho' and 'Choctaw' berries.

Total anthocyanin was positively correlated to ORAC (r=0.64), while total phenolics were not (r=0.03). Total anthocyanins and phenolics were not significantly correlated (r=-0.17).

Kalt et al. (1999) reported that ORAC was increased in strawberry, raspberry, and blueberry when fruit were stored 4-8 days at 10 to 30^{o}C. However, ORAC in these fruit changed little when held at 0 ^{o}C. Blackberries held at 2 ^{o}C are consistent with these results. The ability of blackberries to gain ORAC after a subsequent 2 days storage at 20^{o}C probably depends on the anthocyanin synthesis of the cultivar.

CONCLUSIONS

Storage of erect-type blackberries had inconsistent results on antioxidant content. ORAC tended to decrease rather than increase with storage, while total phenolics increased or stayed the same. Total anthocyanin increased only in stored 'Navaho' berries.

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Tables

Table 1. Main effects on total anthocyanins, phenolics, and ORAC of fresh erect-type blackberries.

	Total anthocyanin (mg cyanidin-3-glucoside/100 g)	Total phenolics (mg gallic acid/g dwt)	ORAC (μmol Trolox eq/100 g dwt)
Storage Temperature (T)			
None	135.3a	274.7b	3340.2a
2 C	128.4a	320.1a	3012.6b
2 C + 20 C	129.3a	325.6a	3208.0ab
Harvest date (HD)			
early season	123.7a	296.5a	2948.2b
mid season	132.8a	311.0a	3277.1a
late season	135.6a	296.8a	3296.5a
Berry color (COL)			
Shiny black	127.5a	310.2a	3228.5a
Dull black	135.4a	294.1a	3189.1a
Cultivar (CV)			
Arapaho	131.8c	307.4c	3512.4b
Choctaw	110.0d	348.5b	3059.9cd
Navaho	148.2b	259.2d	3402.5bc
Shawnee	134.6c	301.0c	2739.6d
*Kiowa	177.4a	397.3a	4048.5a
Interactions			
CV x COL	**	ns	ns
CV x HD	ns	ns	ns
CV x T	*	**	*
COL x HD	ns	ns	ns
COL x T	ns	ns	ns
HD x T	ns	ns	ns

*Kiowa not stored; means not included in other main effects

Means separated within column and category by LSD, $P<0.05$

Table 2. Interaction of cultivar and storage temperature on antioxidant content of erect type blackberries.

Cultivar	Storage temperature	Total anthocyanin	Total phenolics	ORAC
		mg/ 100 g dwt	mg/ 100g dwt	µmol Trolox eq./100 g dwt
Arapaho	Fresh	144.7	289.1	3750
	Stored	119.0*	323.4*	3294*
Choctaw	Fresh	113.9	358.6	3781
	Stored	107.4	340.7	2821*
Navaho	Fresh	136.0	260.1	3235
	Stored	158.4*	258.9	3536
Shawnee	Fresh	138.6	175.3	2691
	Stored	128.4	364.6*	2764

*Means within a cultivar significantly different, t-test, $P<0.05$